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APPLICATION NO.	FILING DATE	FIRST NAMED INVENTOR	ATTORNEY DOCKET NO.
08/963,288	11/03/97	NORSTEDT G	10806-48

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EXAMINER	
BAKER, A	
ART UNIT	**PAPER NUMBER**
1632	7

DATE MAILED: 02/25/99

Please find below and/or attached an Office communication concerning this application or pr ceeding.

Commissioner of Patents and Trademarks

Office Action Summary	Application No. 08/963,288	Applicant(s) Norstedt et al.
	Examiner Anne-Marie Baker, Ph.D.	Group Art Unit 1632

☐ Responsive to communication(s) filed on _____.

☐ This action is **FINAL**.

☐ Since this application is in condition for allowance except for formal matters, **prosecution as to the merits is closed** in accordance with the practice under *Ex parte Quayle*, 1935 C.D. 11; 453 O.G. 213.

A shortened statutory period for response to this action is set to expire *3* month(s), or thirty days, whichever is longer, from the mailing date of this communication. Failure to respond within the period for response will cause the application to become abandoned. (35 U.S.C. § 133). Extensions of time may be obtained under the provisions of 37 CFR 1.136(a).

Disposition of Claims

☒ Claim(s) *1-18* is/are pending in the application.

Of the above, claim(s) _____ is/are withdrawn from consideration.

☐ Claim(s) _____ is/are allowed.

☒ Claim(s) *1-18* is/are rejected.

☐ Claim(s) _____ is/are objected to.

☐ Claims _____ are subject to restriction or election requirement.

Application Papers

☐ See the attached Notice of Draftsperson's Patent Drawing Review, PTO-948.

☐ The drawing(s) filed on _____ is/are objected to by the Examiner.

☐ The proposed drawing correction, filed on _____ is ☐approved ☐disapproved.

☐ The specification is objected to by the Examiner.

☐ The oath or declaration is objected to by the Examiner.

Priority under 35 U.S.C. § 119

☐ Acknowledgement is made of a claim for foreign priority under 35 U.S.C. § 119(a)-(d).

☐ All ☐ Some* ☐ None of the CERTIFIED copies of the priority documents have been

☐ received.

☐ received in Application No. (Series Code/Serial Number) _____.

☐ received in this national stage application from the International Bureau (PCT Rule 17.2(a)).

*Certified copies not received: _____.

☐ Acknowledgement is made of a claim for domestic priority under 35 U.S.C. § 119(e).

Attachment(s)

☒ Notice of References Cited, PTO-892

☒ Information Disclosure Statement(s), PTO-1449, Paper No(s). *5 and 6*

☐ Interview Summary, PTO-413

☐ Notice of Draftsperson's Patent Drawing Review, PTO-948

☐ Notice of Informal Patent Application, PTO-152

--- SEE OFFICE ACTION ON THE FOLLOWING PAGES ---

DETAILED ACTION

Claims 1-18 are pending in the instant application.

Priority

Applicant has not complied with one or more conditions for receiving the benefit of an earlier filing date under 35 U.S.C. 120 as follows:

An application in which the benefits of an earlier application are desired must be copending with the prior application or with an application similarly entitled to the benefit of the filing date of the prior application.

The instant Application claims benefit as a continuation of International Application No. PCT/SE95/01235 filed October 19, 1995 assigned U.S. Serial No. 08/809,256. Application Serial No. 08/809,256 was abandoned on 7/1/97. The instant Application was filed on 11/3/97. Since the applications were not copending, the instant application is not entitled to the benefit of the earlier filing date.

Acknowledgement is made of applicant's claim for priority under 35 U.S.C. 119(a)-(d) based upon Swedish Application No. 9403613-4 filed October 21, 1994. A claim for priority under 35 U.S.C. 119(a)-(d) cannot be based on said application, since the United States application was filed more than twelve months thereafter.

Claim Rejections - 35 USC § 101

35 U.S.C. 101 reads as follows:

> Whoever invents or discovers any new and useful process, machine, manufacture, or composition of matter, or any new and useful improvement thereof, may obtain a patent therefor, subject to the conditions and requirements of this title.

Claims 11, 16, and 17 are rejected under 35 U.S.C. 101 because the claimed invention is directed to non-statutory subject matter. The claims are drawn to a eukaryotic host cell which reads on a host cell *in vivo*, which is non-statutory subject matter. Use of the phrase "isolated eukaryotic host cell" would be remedial.

Claim Rejections - 35 USC § 112

The following is a quotation of the first paragraph of 35 U.S.C. 112:

> The specification shall contain a written description of the invention, and of the manner and process of making and using it, in such full, clear, concise, and exact terms as to enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the same and shall set forth the best mode contemplated by the inventor of carrying out his invention.

Claims 1, 2, 5-11 and 15-17 are rejected under 35 U.S.C. 112, first paragraph, because the specification, while being enabling for a method of enhancing transcription *in vitro* using an SPI-growth hormone responsive element (SPI-GHRE) and lactogenic stimuli, does not reasonably provide enablement for the claimed enhancer element, any method of enhancing transcription *in vivo*, or a method of enhancing transcription *in vitro* or *in vivo* using any enhancer element comprising the nucleotide sequence TTC TGA GAA and exposing the DNA construct to lactogenic stimuli. The specification does not enable any person skilled in the art to which it pertains, or with which it is most nearly connected, to make and use the invention commensurate in scope with these claims.

The specification fails to provide an enabling disclosure for the method of enhancing transcription *in vitro* using any enhancer element comprising the nucleotide sequence TTC TGA GAA because one cannot

predict whether any and all enhancers comprising this nucleotide sequence would be responsive to lactogenic stimuli. Example 1 of the instant specification indicates that Applicants have demonstrated that the core SPI-GAS sequence, TTC TGA GAA, functions as a growth hormone (GH) regulated DNA element *in vitro* when put into a reporter vector. Example 2 indicates that both prolactin and growth hormone activate expression from SPI-TK-reporter gene constructs *in vitro* and that the results obtained with the SPI-GAS element (or SPI-GLE) were similar to the results obtained with the 50 bp SPI-GHRE enhancer. This experiment does not demonstrate that other enhancers that contain the SPI-GAS element would be responsive to lactogenic stimuli.

The specification fails to provide an enabling disclosure for the method of enhancing transcription *in vivo* using any enhancer element comprising the nucleotide sequence TTC TGA GAA because one cannot predict whether results obtained *in vitro* would be obtained *in vivo*. Lavenu et al. investigated the *in vivo* activity of the cis-acting elements known to regulate c-myc expression *ex vivo* and discovered that those elements were not sufficient to direct correct transcription *in vivo* in transgenic mice (abstract). The study strongly suggested that additional regulatory elements located upstream from the promoter and downstream from the polyadenylation site are required to drive correct expression of the c-myc gene. Furthermore, Petitclerc et al., 1995 examined the effect of various introns and transcription terminators on the efficiency of expression vectors in various cultured cell lines and in the mammary gland of transgenic mice and found that transfection experiments, even when stable expression was established were poorly predictive of the potential efficiency of a vector in transgenic animals (abstract). No working examples are provided to demonstrate that the instantly claimed invention can be practiced *in vivo*.

The specification fails to provide an enabling disclosure for an enhancer element responsive to hormonal stimuli, comprising the nucleotide sequence TTC TGA GAA because, as discussed above, there is

no evidence to suggest that any and all enhancers that contain the nucleotide sequence TTC TGA GAA would in fact be responsive to hormonal stimuli. The specification does not offer any guidance for identifying an enhancer element other than SPI-GHRE that is responsive to hormonal stimuli. The existence of other enhancer elements that include the requisite nucleotide sequence has not been demonstrated.

Given the limited *in vitro* working examples, the absence of any *in vivo* working examples, the lack of guidance offered in the specification for identifying the claimed enhancer element, the lack of guidance offered in the specification for methods of enhancing transcription *in vivo*, and the unpredictability of finding other enhancers that contain the requisite nucleotide sequence and are responsive to hormonal stimuli or lactogenic stimuli, one skilled in the art would have been required to have exercised undue experimentation to make and use the claimed invention.

Claims 3, 4, 12-14 and 18 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to enable one skilled in the art to which it pertains, or with which it is most nearly connected, to make and/or use the invention.

Claims 3, 4, 12-14 and 18 are directed to a non-human transgenic mammal having incorporated into its genome a DNA construct comprising a structural gene encoding a desired protein or polypeptide linked to a control sequence for expression in milk-secreting epithelial cells of the mammary gland so that the protein or polypeptide is secreted into the milk, wherein the DNA construct further comprises at least one enhancer element which includes the nucleotide sequence TTC TGA GAA and is responsive to signals generated from prolactine receptors. The claims are also drawn to a method for producing a recombinant protein using the transgenic mammal.

The specification fails to provide an enabling disclosure for the preparation of any species of transgenic mammal of the type claimed because the phenotype of a transgenic animal cannot be predicted and because the *in vivo* expression of a transgene-encoded protein cannot be predicted. The specification does not teach what phenotype would be expected in any species of transgenic mammal, other than the anticipated expression of the transgene. No guidance is provided with respect to how one would have prepared any transgenic animals exhibiting any transgene-dependent phenotypic alteration. No guidance is provided with regard to how the transgene constructs would have been made, introduced into the animal, or expressed in the animal. The mere capability to perform gene transfer in any given species is not enabling for the claimed transgenic mammals because the desired phenotype cannot be predictably achieved simply by introducing transgene constructs of the type recited in the claims. While gene transfer techniques are well-developed for a number of species, especially the mouse, methods for achieving the desired level of transgene expression in appropriate tissues are less well-established. Furthermore, Petitclerc et al., 1995 examined the effect of various introns and transcription terminators on the efficiency of expression vectors in various cultured cell lines and in the mammary gland of transgenic mice and found that transfection experiments, even when stable expression was established, were poorly predictive of the potential efficiency of a vector in transgenic animals. The introduction of DNA into the mammalian genome can ordinarily be achieved most reliably by microinjection or retrovirus-mediated gene transfer. However, the state of the art for transgenics is unpredictable because the method of gene transfer typically relies on random integration of the transgene construct. Insertional inactivation of endogenous genes and position effects (see Wall, 1996, p. 61, paragraph 3) can dramatically influence the phenotype of the resultant transgenic animal. Integration of the transgene near highly active genes or, alternatively, in a transcriptionally inactive region, can influence its level of expression. Furthermore, expression of the transgene and the effect of transgene expression on the

phenotype of the transgenic animal depends on the particular gene construct used, to an unpredictable extent. The particular genetic elements required for appropriate expression varies from species to species. Thus, a construct that confers the desired phenotype in a mouse will not necessarily achieve the same result in a rat. Wall (1996) reports that our lack of understanding of essential genetic control elements makes it difficult to design transgenes with predictable behavior (p. 61, paragraph 3). This is especially relevant for species in which genetic studies are less advanced than in the mouse. Thus, the species-specific requirements for transgene design introduces an additional level of unpredictability associated with the development of transgenic animals. Furthermore, there are inherent physiological differences between mice, goats, cows, sheep, etc. that can affect the phenotype in an unpredictable manner. Absent any specific guidance and specific working examples, the existence of any phenotypic alteration resulting from the introduction of an exogenous gene in conjuction with the claimed enhancer element in any species of mammal, is highly unpredictable. Given the limited working examples and the unpredictability in the art, one of ordinary skill in the art would have been required to engage in undue experimentation in order to make and use the claimed transgenic mammals.

Given that specific phenotypic alterations cannot be predictably achieved by merely transferring a gene of interest into an animal, specific guidance must be provided in the disclosure to enable the instant invention. The specification must teach those skilled in the art how to make and use the full scope of the claimed invention without undue experimentation. The claims cover any transgenic mammal harboring any gene of interest under the regulation of any enhancer of the type claimed from any source, but the specification does not enable such animals nor the use of such animals. In the absence of disclosure of a transgenic mammal, exhibiting the appropriate phenotype, undue experimentation would have been required to make and use the claimed animals.

Claims 3, 4, 9, 10, 12-14 and 18 are rejected under 35 U.S.C. 112, first paragraph, as containing subject matter which was not described in the specification in such a way as to reasonably convey to one skilled in the relevant art that the inventors, at the time the application was filed, had possession of the claimed invention. Applicants are referred to the interim guidelines on written description published June 15, 1998 in the Federal Register at Volume 63, Number 114, pp. 32639-32645 (also available at www.uspto.gov).

Claims 3, 4, 12-14 and 18 are directed to a non-human transgenic mammal having incorporated into its genome a DNA construct comprising a structural gene encoding a desired protein or polypeptide linked to a control sequence for expression in milk-secreting epithelial cells of the mammary gland so that the protein or polypeptide is secreted into the milk, wherein the DNA construct further comprises at least one enhancer element which includes the nucleotide sequence TTC TGA GAA and is responsive to signals generated from prolactin receptors. The claims are also drawn to a method for producing a recombinant protein using the transgenic mammal. However, the specification does not disclose the claimed subject matter at all. There is no written description of the claimed transgenic mammal or the method for using the mammal to produce recombinant protein. Thus the written description is not sufficient to inform a skilled artisan that Applicants were in possession of the claimed invention at the time of filing.

The specification does not contain a written description of any species of transgenic mammal of the type claimed. No particular phenotype is disclosed for the claimed transgenic mammals other than the anticipated expression of the transgene and secretion of the transgene-encoded protein into the milk. There is no demonstration that the claimed mammals would in fact express the transgene from the constructs contemplated at a level sufficient to produce the desired phenotype. Without knowing the phenotype of the transgenic mouse, pig, goat, sheep, cow, etc., one of skill in the art would not know how to use the animal.

Claims 9 and 10 are directed to an expression vector that contains a mammary tissue specific promoter. The specification does not teach or point to any particular mammary tissue specific promoter known in the art. Although mammary tissue specific promoters are known in the art, the specification does not contain a written description of the claimed expression vector. The specification does not disclose the claimed subject matter at all. Thus the written description is not sufficient to inform the skilled artisan that Applicants were in possession of the claimed invention at the time of filing.

The following is a quotation of the second paragraph of 35 U.S.C. 112:

> The specification shall conclude with one or more claims particularly pointing out and distinctly claiming the subject matter which the applicant regards as his invention.

Claims 1-6, 8, 10, 12-14, and 18 are rejected under 35 U.S.C. 112, second paragraph, as being indefinite for failing to particularly point out and distinctly claim the subject matter which applicant regards as the invention.

Claims 1, 2, and 6 are indefinite in their recitation of "lactogenic stimuli" because the specification does not define or describe "lactogenic stimuli." The specification discloses that the enhancer element confers a response to signals evoked by pituitary hormones belonging to the group of lactogenic hormones such as prolactin and placenta lactogen. However, the specification does not clearly indicate whether the term "lactogenic stimuli" as used in the claims refers only to the two hormones mentioned, or encompasses other lactogenic hormones.

Claims 2, 4, 5, 8, and 10 are indefinite in their recitation of the "SPI-GHRE" element because it is not clear what the actual boundaries of the element are. On p. 2 a 50 bp sequence is identifed as the SPI-GHRE but it is unclear as to whether the SPI-GHRE constitutes this entire sequence. The Yoon et al.

reference defines the regulatory element up to a point, but then does not test smaller fragments for activity. Thus, it is not evident how much of the 50 bp sequence actually constitutes the responsive element.

Claim 3 is indefinite with respect to the method step that involves "providing the nucleotide sequence TTC TGA GAA in an expression vector in the non-human mammal" because it is unclear how the expression vector is being provided to the animal. The specification does not discuss any methods for delivering an expression vector to an animal.

Claim 12, 13, 14, and 18 are indefinite in their recitation of a "control sequence" for expression in milk-secreting epithelial cells of the mammary because it is unclear whether the term "control sequence" refers to a promoter, a tissue-specific enhancer, or some other genetic element.

Claim Rejections - 35 USC § 102

The following is a quotation of the appropriate paragraphs of 35 U.S.C. 102 that form the basis for the rejections under this section made in this Office action:

> A person shall be entitled to a patent unless --
>
> (b) the invention was patented or described in a printed publication in this or a foreign country or in public use or on sale in this country, more than one year prior to the date of application for patent in the United States.

While the specification does not explicitly define "lactogenic stimuli," it is known in the art that human growth hormone can bind to both lactogenic and somatogenic receptors with high affinity (Le Stunff et al., 1996). Therefore, growth hormone is considered to be included as "lactogenic stimuli."

Claims 1, 2, 9, 10, 16, and 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Yoon et al., 1990.

The claims are directed to a method of enhancing the transcription of a gene in a DNA construct incorporated into the genome of a eukaryotic host cell, wherein the DNA construct comprises a structural gene for a desired protein or polypeptide and a promoter upstream of the structural gene, wherein the method comprises providing upstream of the promoter at least one enhancer element comprising the nucleotide sequence TTC TGA GAA, and exposing the DNA construct to lactogenic stimuli. The claims are particularly directed to the method of enhancing transcription, wherein the enhancer element is the serine protease inhibitor (SPI) growth hormone responsive element. The claims are also drawn to an expression vector comprising an enhancer element including the nucleotide sequence TTC TGA GAA, particularly the SPI-growth hormone responsive element. Also claimed are eukaryotic host cells containing the expression vector.

Yoon et al. reported that transcription of the serine protease inhibitor (SPI) 2.1 gene is induced by growth hormone in rat liver. Yoon et al. isolated and characterized the SPI 2.1 gene from a rat genomic library and examined the 5'-flanking region of the gene which revealed a Dnase I hypersensitive site within 500 base pairs of the transcriptional inititiation site. Portions of the 5'-flanking region were fused to a heterologous promoter and reporter gene and introduced into primary rat hepatocytes by lipofection, thereby generating expression vectors and eukaryotic host cells as instantly claimed (p. 19948, column 2). SPI 2.1 sequences from -275 to -54 gave a 2-3-fold induction of reporter gene activity in cells grown in the presence of GH, similar to the level of induction of the endogenous SPI 2.1 mRNA in isolated hepatocytes. Thus, the instantly claimed method of enhancing transcription was demonstrated for the disclosed DNA constructs by measuring induced reporter gene activity. Further definition of the essential sequences revealed that a

segment from -147 to -102 could confer GH responsiveness when linked in tandem copies in front of a heterologous promoter. Using the gel shift assay, a nuclear factor from normal rat liver was identified which could interact with this minimal response fragment. GH regulation of this activity was suggested by the fact that it was absent in hypophysectomized animals but reappeared 1 hour after treatment of said animals with GH.

Claims 1, 2, 5-11, 15, 16, and 17 are rejected under 35 U.S.C. 102(b) as being anticipated by Sliva et al., 1994.

The claims are drawn to a method of enhancing transcription, an enhancer element comprising the nucleotide sequence TTC TGA GAA, an expression vector comprising the enhancer element, and a eukaryotic host cell containing the expression vector.

Sliva et al. identified a 9-base pair DNA element, TTC TGA GAA (p. 26208, column 2, paragraph 1), the SPI-GLE 1, which forms a complex with nuclear proteins following activation by growth hormone and which, when placed upstream of a minimal thymidine kinase promoter, drives chloramphenicol acetyltransferase expression in a growth hormone-dependent fashion (Abstract and p. 26209, "Assembly of Reporter Constructs"). Competition studies with oligonucleotides similar to the SPI-GLE 1 reveal the sequence of a consensus element that specifically binds growth hormone-regulated nuclear proteins. Thus, the claimed method of enhancing transcription, the enhancer element, the expression vector, and the eukaryotic host cell containing the expression vector are all disclosed by Sliva et al.

Claims 1 and 2 are rejected under 35 U.S.C. 102(b) as being anticipated by Le Stunff et al., 1996.

The claims are directed to a method of enhancing transcription.

Le Stunff et al. reveal that prolactin induced nuclear protein binding to the GH-responsive element of the serine protease inhibitor 2.1 promoter and activated SPI 2.1 gene expression *in vivo* (Abstract).

The prior art does not teach or fairly suggest making transgenic mammals harboring a transgene construct containing an enhancer element comprising the nucleotide sequence TTC TGA GAA, using the mammals to produce recombinant protein, or otherwise providing an expression vector of the type claimed to a mammal to produce recombinant protein. As discussed above the enhancer element was known in the prior art and its responsiveness to growth hormone and prolactin was known. However, transgenic mammals harboring a construct containing the enhancer element, wherein the mammals expressed a recombinant protein in their milk, were not disclosed in the prior art.

Any inquiry concerning this communication or earlier communications from the examiner should be directed to Anne-Marie Baker whose telephone number is (703) 306-9155. The examiner can normally be reached Monday through Friday from 8:00 AM to 5:30 PM.

If attempts to reach the examiner by telephone are unsuccessful, the examiner's supervisor, Brian Stanton, can be reached on (703) 308-2801. The fax phone number for the organization where this application or proceeding is assigned is (703) 308-8724.

Any inquiry of a general nature or relating to the status of this application or proceeding should be directed to the receptionist whose telephone number is (703) 308-0196.

Anne-Marie Baker, Ph.D.
February 8, 1999

Bruce Campell

BRUCE R. CAMPELL
PRIMARY EXAMINER
GROUP 1800